The Characterization of Metal Concentration and Distribution in Lichens with Varying Tolerance to Pollution.

A. Carani[1], D. Covelli[1], D. Crossland[1], H. Dudek[1], L. Elias[1], S. Metha[1], S. Metha[1], E. Srivastava[1],
A. Lanzirotti[2], M. Newville[2], R. Winarski[3] and K. Beardsley[1]
[1] Glenbard East High School, Lombard IL, 60148, [2]Center for Advanced Radiation Sources, University of Chicago [3] Advanced Photon Source, Argonne National Laboratory, Lemont, IL 60439

Abstract

Lichens are used for biomonitoring pollution because of their differential ability to survive in polluted environments. Various lichens have been identified as tolerant or intolerant to phytotoxic gases common in air pollution. Lichen tolerance rating is based on their varying growth rates in response to these phytotoxic compounds that can impair function (Huckaby). Lichens are a symbiont organism comprised of a fungus and photobiont (alga or cyanobacterium). Tolerant lichens can sequester metals within crystals in extracellular locations and in fungal tissue in order to protect the photobiont and the survival of the symbiont organism (Bačkor & Loppi, Purvis et. al). Therefore, we would expect differing deposition patterns of metals within the algal and fungal regions of the tolerant and intolerant lichens found in areas of differing pollution. This investigation characterized pollution tolerant *Candelaria concolor*, and pollution intolerant *C. fibrosa*. Synchrotron XRF analysis was used to determine the differences in metal deposition of *C. concolor* collected from areas of high and low phytotoxic pollution and *C. fibrosa* collected from an area of low phytotoxic pollution. There was a tenfold increase in zinc levels in the fungal layer of the tolerant *C. concolor* collected from an area of higher pollution versus *C. concolor* collected from an area of lesser pollution, while zinc concentration in the algal layer of both samples remained the same. This sequestration pattern confirms existing evidence of mechanisms by which tolerant lichens allow for protection of algal cells against zinc toxicity (Purvis). Tolerant *C. concolor* collected from an area of higher pollution had a 100-fold higher level of calcium versus the intolerant *C. fibrosa*. These elevated calcium levels are in agreement with existing evidence that lichen protects against the damaging effects of sulphur dioxide (a phytotoxin) with elevated levels of calcium (Gunathilaka et. al). The images produced from sXRF data visualize this ability of tolerant lichens to offset the toxic effects of zinc in the environment.

Lichen Anatomy

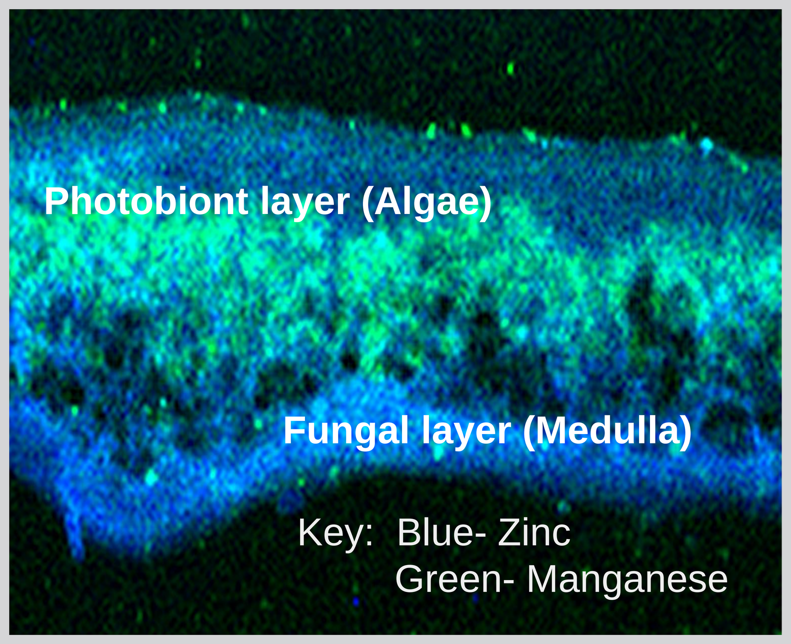

Figure 1: Longitudinal section of *C concolor.* Manganese is sequestered in the algal layer as a part of Photosystem II. The zinc is sequestered in the fungal layer.

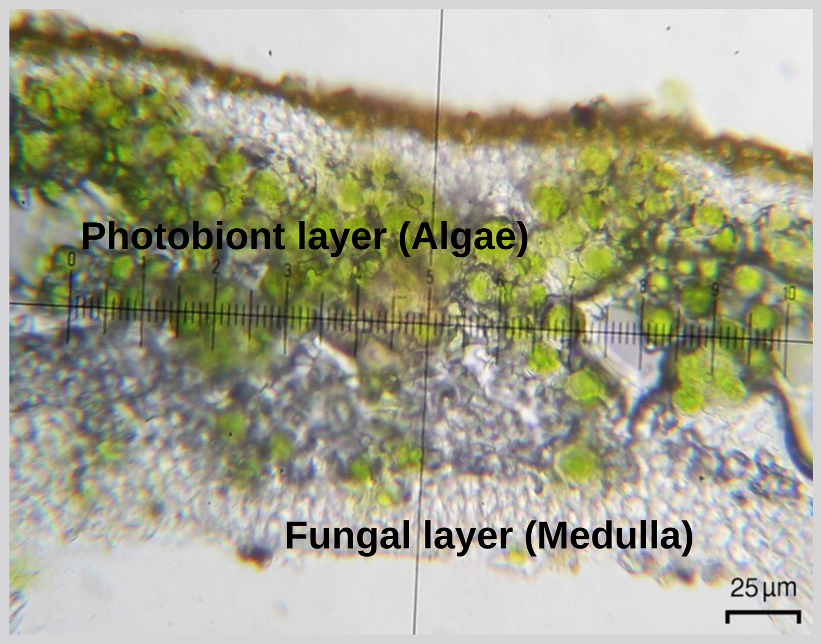

Figure 2: Longitudinal section of *C concolor.* Courtesy of Jason Hollinger.

Methods

Figure 3: Leica cryostat microtome used to section our lichen samples.

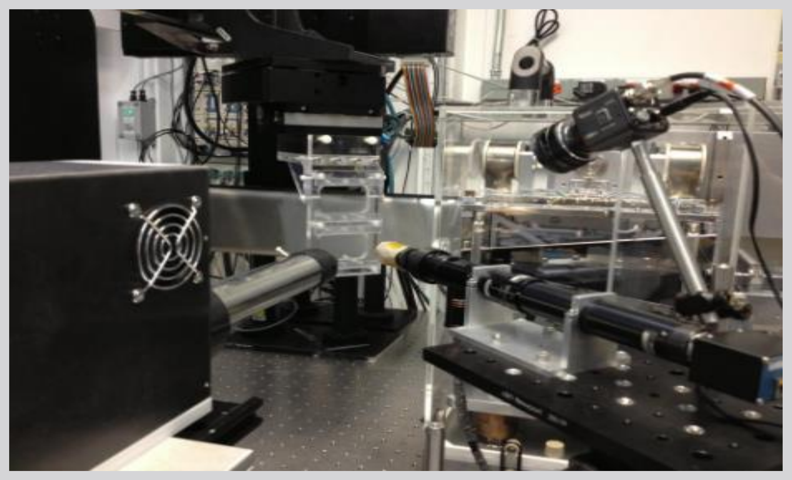

Figure 4: Beamline 13 ID-E at GSECARS.

- Lichen samples were cryogenically frozen in the Leica Biosystems Tissue Freezing Medium
- Lichen samples were sectioned into longitudinal segments of 30 microns by Leica's cryostat microtome, then placed on Kapton film
- Elemental analysis of the sections was accomplished via X-ray fluorescence using a 4-element silicon drift detector with an incident beam energy of 12KeV

Conclusions

- The tolerant species sequester metals in higher amounts when compared with the intolerant species. The tolerant species is able to continue biologic functions in higher levels of metals versus the intolerant species (Figure 5).
- By sequestering toxic levels of zinc in the fungal-medulla layer the organism ensures protection of algal layer photosynthetic biochemistry (Figure 7). The intolerant species does not show this pattern of sequestration, thus intolerance to increased levels of pollution may be due to its inability to immobilize metals in the medulla (Figure 8).
- The tolerant species from an area of greater pollution sequesters 10x greater levels of metals versus the tolerant species from an area of lesser pollution. One hypothesis explaining the higher levels of calcium, titanium, and iron is that the metals entered the lichen as airborne dust from roadways, automotive exhaust, and industry. We hypothesize that this ability to immobilize metals at increasing levels gives the lichen tolerance to increasing levels of environmental pollution (Figure 6).

Results

Tolerant vs. Intolerant Species

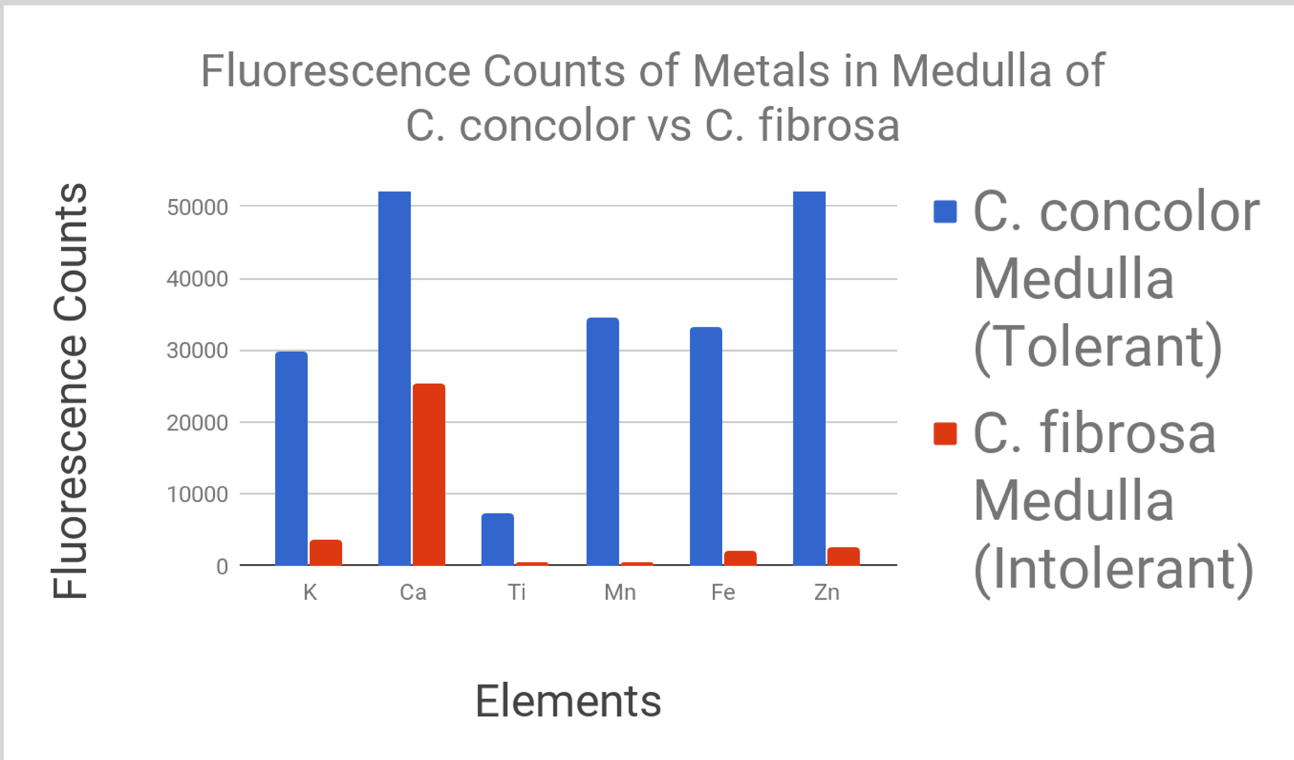

Figure 5: Tolerant species sequester metals more effectively than intolerant species varying from 10-100x greater.

Effects of Greater Pollution

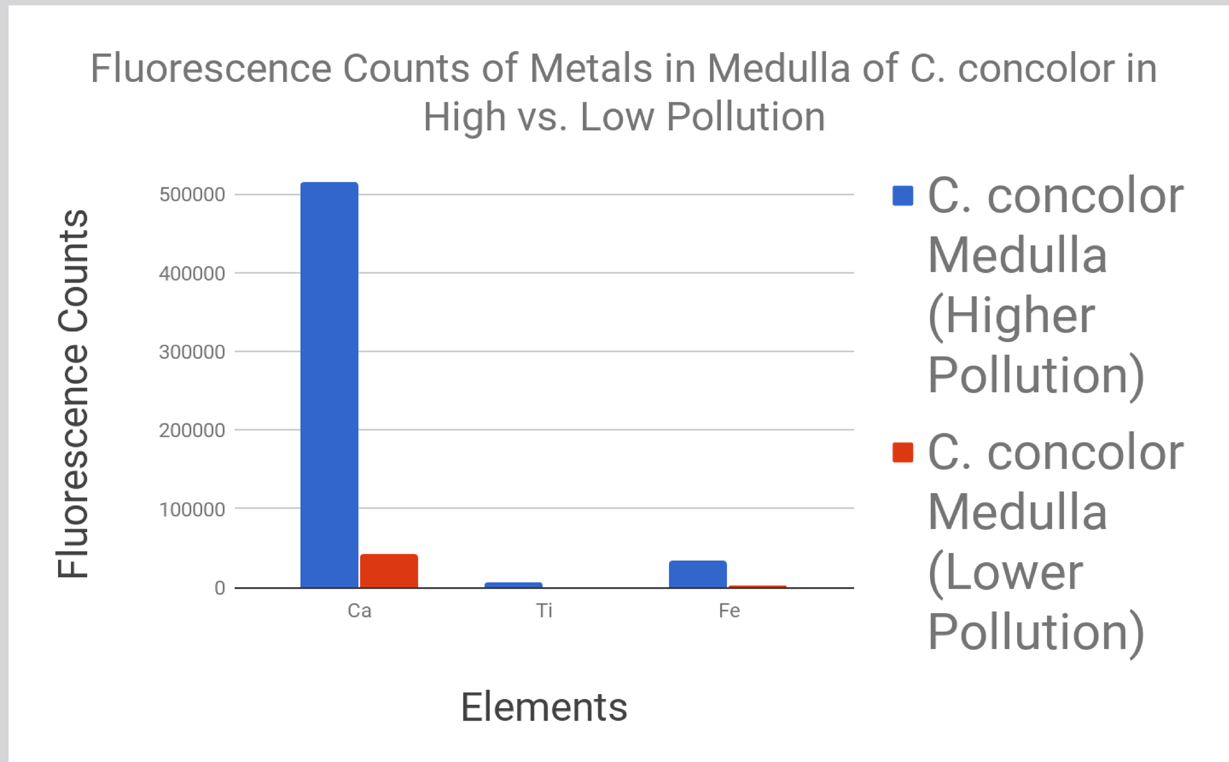

Figure 6:*C. concolor* from areas of higher pollution have concentrations of calcium, titanium, and iron 10x greater than *C. concolor* from areas of lower pollution.

Distribution of Zinc in Lichen Tissue

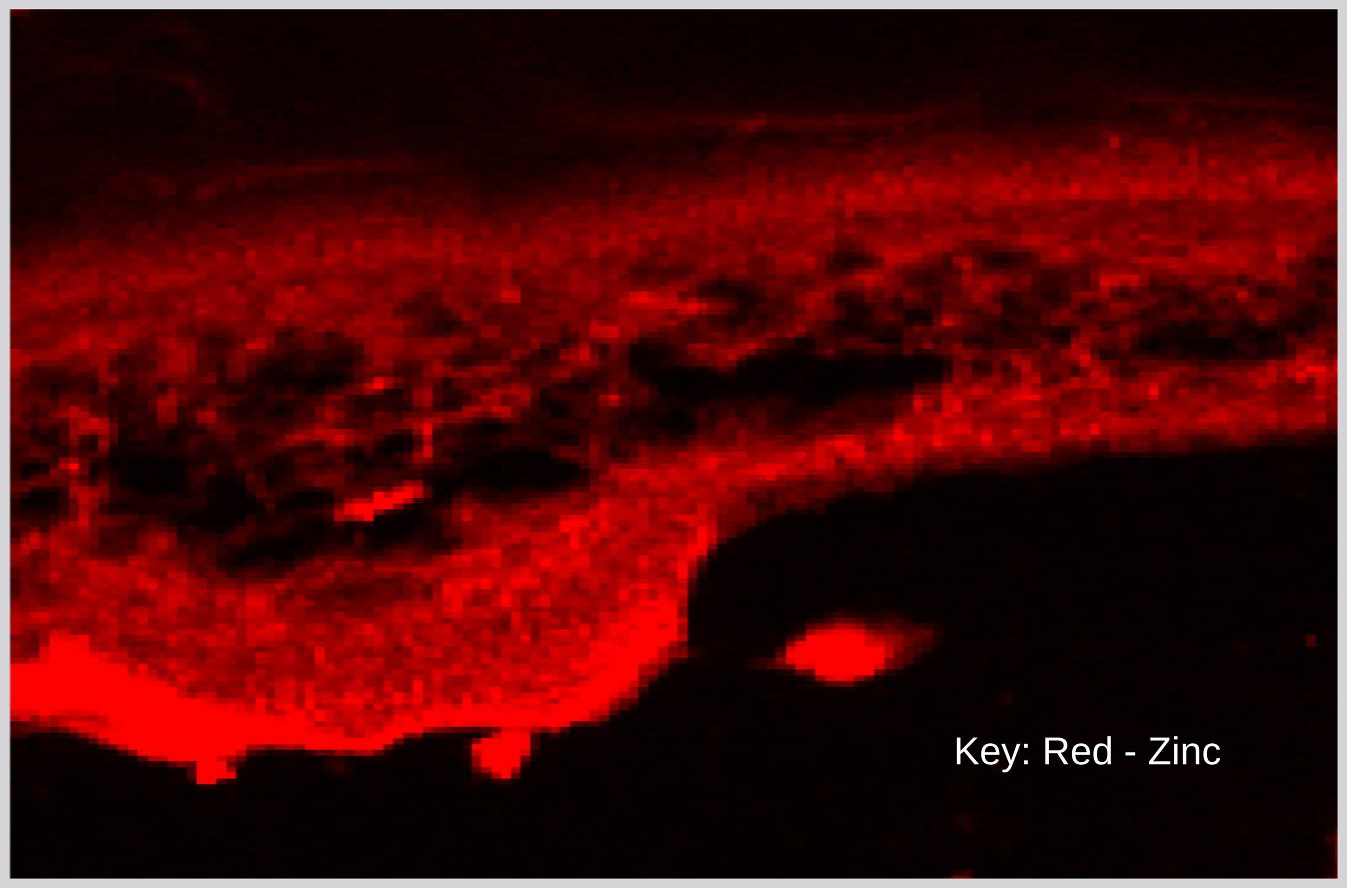

Figure 7: Section of *C. concolor.* The tolerant species sequesters zinc in the medulla. Zinc is represented by the red deposition pattern.

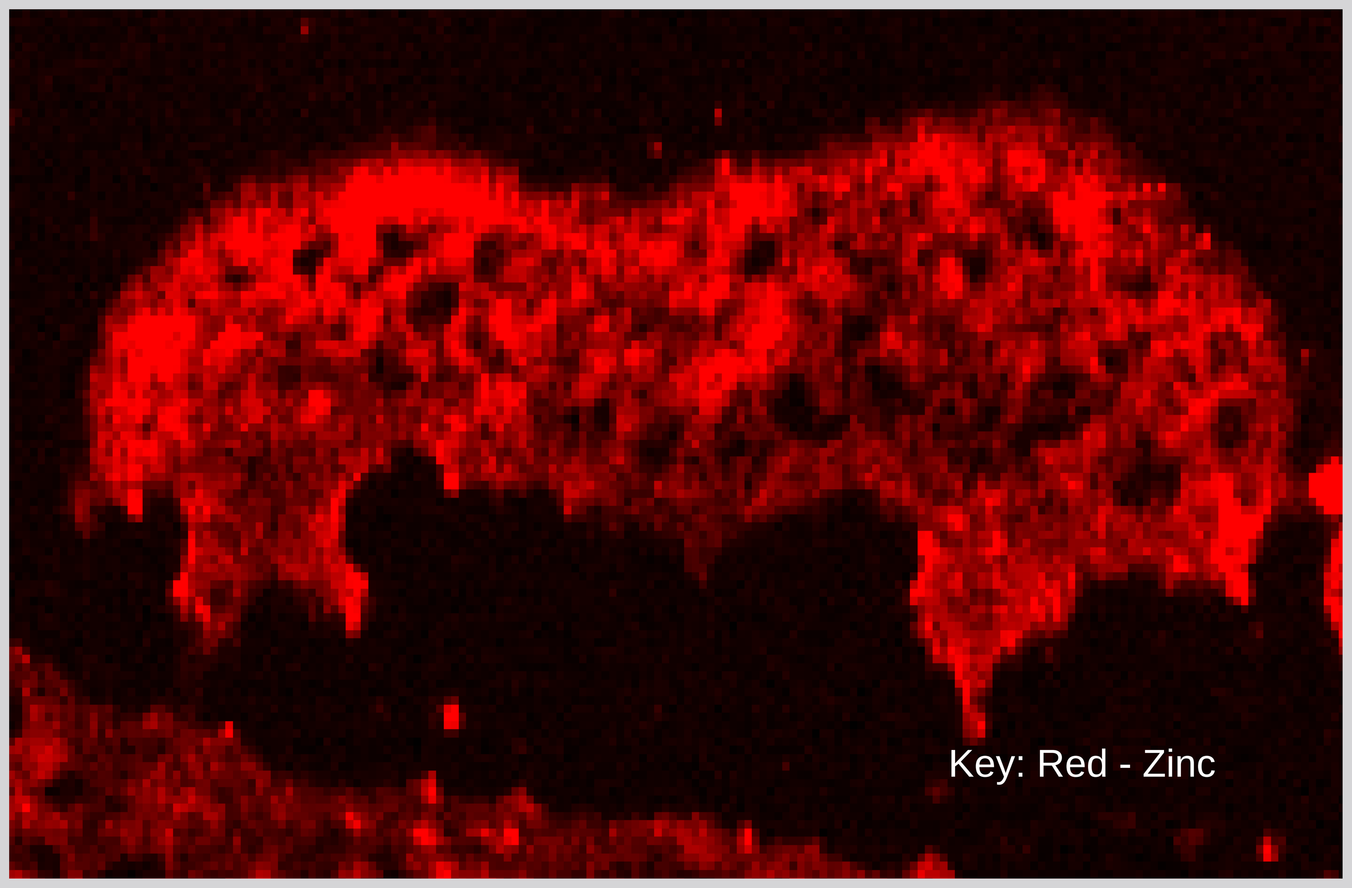

Figure 8: Section of *C. fibrosa.* Zinc is equally distributed throughout the algal and medulla layers in the intolerant species.

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